

Multiplication of *Staphylococcus aureus* in Synthetic Cream Fillings and Pies

FRANCIS D. CRISLEY, Ph.D., ROBERT ANGELOTTI, Ph.D., and MILTON J. FOTER, Ph.D.

RECENTLY a number of prepared commercial cream fillings described as "synthetic" have been made available to bakeries in various parts of the United States under the claim that these fillings are not capable of supporting bacterial growth and that pies made with such fillings can be marketed without refrigeration. Numerous queries received by the Public Health Service from health departments indicated the necessity of testing these products in order to determine whether they present a potential health hazard when held at room temperature.

The synthetic cream fillings are marketed as dry, powdered preparations containing no milk, eggs, or shortening, and are thus devoid of some ingredients that ordinarily make cream-filled products good media for bacterial growth. The ingredients of the fillings tested, as given on the manufacturer's label on each package, are listed in table 1. A synthetic cream filling consists basically of starch, sugars (sucrose and dextrose), sodium chloride, natural and artificial flavorings, emulsifiers and stabilizers, preservatives, and other chemical additives. As indicated in table 1, the pH of all cream fillings tested, except filling F, was found to be either acid or alkaline. Water is used to rehydrate all fillings except F, for which milk is recommended.

Methods of Investigation

Samples of cream fillings used in this study were supplied by the bureau of food and environmental sanitation, Cincinnati Health Department; the Food Sanitation Section, Milk and Food Branch, Public Health Service, Washington, D.C.; the Milk and Food Section, Public Health Service Region IX, San Francisco,

Calif.; and the office of Public Health Service Region II, New York, N.Y. Gayle Mulberry and Nancy Batterson, microbiologists, Food Microbiology Section, Robert A. Taft Sanitary Engineering Center, assisted in carrying out the laboratory procedures.

Preparation of inoculum. An enterotoxigenic culture of *Staphylococcus aureus* 196E was employed. It was inoculated by the deep stabbing technique and maintained at room temperature on a semisolid stock culture medium, in use at the University of Kentucky, that contains proteose peptone No. 3 (Difco), 10 gm.; dextrose, 1 gm.; Na₂HPO₄ (anhydrous), 1.5 gm.; NaCl, 5 gm.; gelatin (bacteriological), 10 gm.; and agar, 5 gm. All dry ingredients except agar were dissolved in 1,000 ml. of distilled water, and the reaction was adjusted to pH 7.4. The agar was added and dissolved by bringing the solution to the boiling point. The medium was then tubed for use in deep agar cultures, 8 ml. per test tube, and sterilized by autoclaving for 15 minutes at 121° C.

Standardization of the optical density of the suspension of *S. aureus* in phosphate-buffered dilution water (1) with a pH of 7.0 had previously been carried out in relation to viable plate counts on trypticase soy agar, and the method of preparation of the inoculum was exactly like the standardized procedure. A primary transfer from stock was made into a tube of trypti-

All the authors are with the Robert A. Taft Sanitary Engineering Center, Public Health Service, Cincinnati, Ohio. Dr. Crisley is research microbiologist in the Food Microbiology Section of which Dr. Angelotti is chief. Dr. Foter is assistant chief of Milk and Food Research.

case soy broth that was incubated for 24 hours at 35° C. Portions of the resulting broth culture were used to seed the surface of trypticase soy agar slopes in dilution bottles. These agar cultures were also incubated for 24 hours at 35° C. Bacterial growth was washed off the agar with small volumes of standard buffered dilution water (1) and centrifuged at 3,200 rpm in an International refrigerated centrifuge at 10° C. The cell suspension was washed twice more in succession to remove traces of adhering media that might provide growth materials for the multiplication of the organisms after they were added to cream fillings. The optical density was adjusted by spectrophotometer to a selected percent transmittance at 620 millimicrons, and a viable plate count of the suspension was made. The desired cellular concentration used for inoc-

ulation of the fillings was made by preparing appropriate dilutions of the adjusted suspensions in standard phosphate-buffered dilution water (1).

Preparation of cream fillings. Fillings were prepared according to manufacturer's instructions on each package. In preparing fillings A, B, C, D, E, and G, a recommended sugar solution was brought to a boil and the filling mixture was added. The mixtures for A and D were then cooked until thick with constant stirring. Mixtures for B, C, and G were brought to a second boil with constant stirring. All heated mixtures were cooled to permit further gelling before inoculation with cultures or preparation of pies. Fillings E and F were simply rehydrated, E with cold water and F with cold milk, except for the experiment summarized in

Table 1. Ingredients in seven commercial synthetic cream fillings as stated on labels and pH of each filling

Ingredient	Cream filling						
	A ¹	B ²	C ²	D	E ³	F ^{3,4}	G
Starch:							
Unspecified or blended.....	x	x	x		x		
Precooked.....						x	
Corn.....				x			x
Tapioca.....							x
Sugar:							
Dextrose.....						x	x
Sucrose presumably, stated as sugar.....	x	x	x		x	x	
Salt.....	x	x	x	x	x	x	x
Coloring:							
Artificial.....		x	x		x		x
U.S. certified.....				x		x	
Pure food.....	x						
Flavoring:							
Artificial or imitation.....	x	x	x	x	x	x	
Natural.....	x						
Imitation vanilla.....							x
Vanilla.....						x	
Other additives:							
Calcium acetate.....						x	
Calcium gluconate.....					x		
Carboxymethylcellulose.....							x
Cellulose gum.....	x						
Sodium phosphate.....					x	x	
Sodium propionate.....	x		x		x		x
Trisodium phosphate.....							x
Vegetable emulsifier.....					x		
Vegetable gum.....							x
Vegetable stabilizer.....					x		
pH ⁵	8.5	5.0	5.85	5.65	8.1	6.6	9.0

¹ Also marketed in a recipe that contains sodium benzoate instead of sodium propionate.

² Identical products of the same manufacturer, except that recipe C contains sodium propionate.

³ Product does not require cooking, heating, or "bringing to the boiling point."

⁴ Manufacturer's instructions specify rehydration of the product with fresh or reconstituted dry milk.

⁵ When prepared according to manufacturer's directions.

table 2 in which water was employed. Products were rehydrated with unsterile distilled water; clean, unsterile utensils were used under ordinary kitchen conditions. Cream fillings were not sterilized in order to avoid physical or chemical alterations in the products that would not occur in actual baking practice and also to observe the effect of the normal filling flora on added staphylococci. After preparation, the rehydrated fillings were handled aseptically. They were weighed into sterile, wide-mouthed, screw-capped jars, 100 grams per jar. One milliliter of the standardized inoculum was added and the contents were thoroughly mixed with a sterile tongue depressor. Uninoculated controls were also prepared.

Preparation of completed pies. Baked pie shells were filled with prepared cooled cream fillings to produce completed pies according to common baking practice for these products, which are not heated after filling or are only heated superficially to brown the surface of the filling material. A commercially prepared pie crust mix sold in stick form was employed. This mix contained flour, shortening with freshness preserver, salt, and artificial color and was prepared by crumbling the stick into a bowl, adding boiling water, and mixing until the dough could be formed. The dough was rolled out to the desired size and placed in a wide-mouth, screw-capped food jar. The sides of the crust were made to extend about 1½ inches above the bottom. The pie shell was baked in

a preheated oven at 450° F. for 10 minutes. Shells were allowed to cool and a 200-gm. portion of inoculated or uninoculated synthetic filling was aseptically added to each shell to within ¼ to ½ inch of the top.

Preparation of media. Trypticase soy agar was employed for total bacterial counts of uninoculated fillings and pies. Counts of staphylococci were made on a tellurite-polymyxin-egg yolk agar medium (TPEY) which was developed in this laboratory for use with foods and has thus far proved to be superior to other media. TPEY basal medium is composed of Bacto-tryptone, 10 gm.; Bacto yeast extract, 5 gm.; d-mannitol, 5 gm.; NaCl, 20 gm.; LiCl, 2 gm.; and distilled water, 900 ml. The ingredients were dissolved by warming, the reaction was adjusted to pH 7.2 to 7.3 electrometrically, and 18 gm. of Bacto agar was added and dissolved by heating. The basal medium was autoclaved at 121° C. for 15 minutes and allowed to cool to 50° to 55° C. in a water bath. The remainder of the ingredients were added aseptically to the cooled medium. Egg yolk emulsion (30 percent volume per volume) in physiological saline was made by soaking fresh eggs for about 1 minute in a 1:1,000 dilution of saturated HgCl₂ solution. They were aseptically cracked, the yolks were "kitchen separated" from the whites and were blended in a Waring Blendor with the required amount of saline for about 5 seconds. One hundred milliliters of this emulsion was added to 900 ml. of

Table 2. Multiplication of *Staphylococcus aureus* and normal bacterial flora of synthetic cream fillings made with water

Cream filling	Bacterial counts per gram after incubation at room temperature					
	Uninoculated		Inoculated with staphylococci			
	0 hours	72 hours	0 hours	24 hours	48 hours	72 hours
A-----	1.9 x 10 ⁸	2.5 x 10 ⁵	1.2 x 10 ⁸	3.3 x 10 ²	*0	*0
B-----	28	1.3 x 10 ⁴	9.4 x 10 ²	3.1 x 10 ²	2.0 x 10 ¹	*0
C-----	18	7.6 x 10 ⁵	1.1 x 10 ⁸	4.6 x 10 ²	6.3 x 10 ²	4.0 x 10 ²
D-----	<1.0	1.8 x 10 ⁵	7.4 x 10 ²	5.9 x 10 ²	1.1 x 10 ³	9.8 x 10 ³
E-----	3.0 x 10 ²	1.6 x 10 ⁷	1.1 x 10 ⁸	7.3 x 10 ⁵	6.2 x 10 ⁶	1.4 x 10 ⁷
F-----	2.0	3.3 x 10 ⁶	1.0 x 10 ⁸	4.2 x 10 ²	3.1 x 10 ²	2.4 x 10 ²
G-----	1.9 x 10 ⁸	2.5 x 10 ⁵	7.7 x 10 ²	1.0 x 10 ³	1.2 x 10 ³	2.2 x 10 ²
Laboratory control custard-----	14.0	4.3 x 10 ⁸	1.3 x 10 ⁸	1.0 x 10 ⁹	1.9 x 10 ⁹	2.0 x 10 ⁹

*No detectable growth in 2.0 ml. of a 1:2 dilution.

the cooled basal medium, followed by 0.4 ml. of a 1 percent Seitz-filtered solution of Polymyxin B (Nutritional Biochemicals, Cleveland, Ohio), to a final concentration of 40 μ g. per ml., and 10 ml. of a sterile 1 percent solution of potassium tellurite (autoclaved separately). The finished medium was then poured into sterile petri dishes, 15 to 17 ml. per plate, and allowed to dry at room temperature before use.

Incubation of fillings and pies. Inoculated and uninoculated pies and fillings were allowed to incubate on the laboratory bench at room temperature (22° to 25° C.) to duplicate holding conditions claimed to be suitable for the products.

Plate counts. Plate counts were made immediately after inoculation (0 time counts) and at 24, 48, and 72 hours, except for counts of uninoculated products, which were made as deemed necessary. Ten-gm. samples of cream filling or 50-gm. wedges of completed pie were diluted decimally with phosphate dilution water (1), the latter by blending the initial 1:10 dilution in a Waring Blendor. The surfaces of duplicate plates of trypticase soy agar and TPEY were seeded with 0.1-ml. portions of each decimal dilution, and the inoculum was spread evenly over the agar surfaces with sterile glass spreader rods. Inoculated plates were allowed to dry and were incubated at 35° C. for 24 hours. Counts were made on a Quebec colony counter.

Laboratory custard. An unsterile control custard filling, made with milk and eggs and prepared under the same conditions as the synthetics, according to the recipe of Angelotti and co-workers (2), was used in the initial studies for comparison with synthetic fillings.

Results and Discussion

Bacterial growth in fillings. Table 2 indicates that total bacterial counts on uninoculated fillings prepared with unsterile distilled water ranged from less than 1 per gram (filling D) to 1,900 per gram (A and G). The count on laboratory custard made with milk and eggs was low (14 per gram). Filling F, the only one usually prepared with milk, also showed a low count (2 per gram). Because previous experiments had shown increases in bacterial counts at 24 and 48 hours, total counts were not

made after these time periods. At the end of 72 hours, the normal flora of the products had all increased by 2 to 6 logarithms, which indicated that the cream fillings, without exception and regardless of their initial bacterial load, were capable of supporting bacterial multiplication at room temperature. Laboratory custard supported the highest growth, yielding an increase of 7 log steps in 72 hours.

Of the fillings seeded with *S. aureus* 196E, decreases in count over the 72-hour period were noted for A, B, C, F, and G, the reductions being quite marked in A and B. The staphylococcal population was maintained at approximately the initial level in C for 24 hours and in G for 48 hours before decreasing slightly. Staphylococcal multiplication occurred in D (1 log step) and E (4 log steps) in 72 hours. Laboratory custard showed an increase in staphylococci of about 6 log steps. It is noteworthy that C, similar in composition to B except that it contained sodium propionate, showed a decrease in staphylococci that was lower than that in B, indicating that the preservative had no effect. Thus, only two of the fillings supported definite staphylococcal multiplication, although three others generally maintained the initial population or showed only slight decreases. The production of enterotoxin in these three products is problematical, but the ability of the fillings to sustain a viable staphylococcal population may be considered as a potential hazard.

Although no marked spoilage of the products was noted after 24 hours, slight off odors were detectable in several fillings, probably as a result of the activities of the saprophytes, mostly bacilli, present in all fillings. Liquefaction in several fillings was apparent within 48 hours and became marked in 72 hours.

Suppression by the concomitant flora is one possible explanation for the limited growth of staphylococci noted in this study. Peterson and co-workers (3) have found such inhibitions to be especially marked at pH levels below 6.0 and above 8.0. Most of the synthetics examined in this study showed pH values in the inhibitory range. Although pH was probably a contributing factor, the pattern of suppression in the cream fillings did not correlate exactly with the pH values reported to be optimal for inhibition of staphylococci by accompanying bacteria (3).

Table 3. Multiplication of *Staphylococcus aureus* in three synthetic cream fillings adjusted to pH 7 with sterile 10 NNaOH solution

Cream filling	Number of staphylococci per gram after incubation of cream filling at room temperature			
	0 hours	24 hours	48 hours	72 hours
A-----	1.0 x 10 ³	*8.0 x 10 ²	*3.3 x 10 ²	(†)
C-----	1.0 x 10 ³	1.4 x 10 ³	*1.0 x 10 ⁴	*9.6 x 10 ³
G-----	8.4 x 10 ²	*1.0 x 10 ³	*1.6 x 10 ³	*1.9 x 10 ³

*Heavy outgrowth of bacilli.

†Not countable because of extremely heavy outgrowth of bacilli.

Growth of staphylococci in neutralized fillings. Table 3 shows that the staphylococcal population of A, the filling in which the pH was adjusted to 7.0, decreased by 1 log step in 48 hours and was completely overgrown by bacilli in 72 hours. Slight increases of staphylococci were apparent in fillings in which the reaction was similarly adjusted in C and G, in spite of heavy outgrowth of bacilli, although these may be considered as little more than maintenance of the original inoculum levels. Nevertheless, in comparison with results in table 2, staphylococci survived in greater numbers in neutralized filling A and multiplied, however slightly, in neutralized C and G, even though outgrowth of saprophytes appeared to be marked in the neu-

tralized fillings and probably was somewhat inhibitory to the staphylococci.

Effect of amount of inoculum on multiplication of staphylococci. Peterson and co-workers (4) have reported that repression of staphylococci by concomitant flora was greater as the proportion of staphylococci to other bacteria grew smaller. Since this point was obviously pertinent to the problem, experiments with staphylococcal inoculums ranging from approximately 10³ to 10⁷ per gram were conducted on synthetics A and C. Table 4 shows that in filling A staphylococci progressively decreased from all the initial levels during the 72-hour period, the rate of decrease being somewhat inversely proportional to the amount of the initial inoculum. In C a slight unexplained increase was consistently noted in replicate experiments at the 10⁵ inoculum level. At all other levels progressive decreases were noted, but the reductions were generally less sharp than in A, especially at the lower levels. In view of the failure of staphylococci to initiate significant multiplication at any of the levels tested, it is probable that other factors, such as nutrition and not simple suppression by concomitant flora, were involved.

Effect of added food substances on bacterial growth. During the initial experiments, our attention was called to the manufacturer's literature which recommended supplementing

Table 4. Effect of size of inoculum on the ability of two synthetic cream fillings to support growth of *Staphylococcus aureus*

Estimated inoculum per gram at 0 hours	Number of staphylococci per gram after incubation of cream filling at room temperature			
	Actual	24 hours	48 hours	72 hours
Cream filling A:				
10 ³ -----	8.4 x 10 ²	2.5 x 10 ²	*186	*†0
10 ⁴ -----	1.1 x 10 ⁴	4.0 x 10 ³	*2.3 x 10 ³	*70
10 ⁵ -----	8.7 x 10 ⁴	4.9 x 10 ⁴	1.5 x 10 ⁵	*4.0 x 10 ³
10 ⁶ -----	9.3 x 10 ⁵	3.9 x 10 ⁵	5.5 x 10 ⁴	3.5 x 10 ³
10 ⁷ -----	9.8 x 10 ⁶	7.6 x 10 ⁶	6.4 x 10 ⁶	1.9 x 10 ⁶
Cream filling C:				
10 ³ -----	1.1 x 10 ³	5.4 x 10 ²	5.5 x 10 ²	4.0 x 10 ²
10 ⁴ -----	9.2 x 10 ³	3.3 x 10 ³	2.0 x 10 ³	1.4 x 10 ³
10 ⁵ -----	1.0 x 10 ⁵	1.6 x 10 ⁵	2.3 x 10 ⁵	4.6 x 10 ⁵
10 ⁶ -----	1.2 x 10 ⁶	4.5 x 10 ⁵	1.7 x 10 ⁵	2.0 x 10 ⁵
10 ⁷ -----	9.7 x 10 ⁶	7.1 x 10 ⁶	8.0 x 10 ⁵	3.0 x 10 ⁵

*Heavily contaminated by outgrowth of bacilli.

†No staphylococci detected in 2.0 ml. of a 1:2 dilution.

several fillings with shortening, sugar, egg white, chocolate, coconut, butterscotch flavoring, fruit, and fruit flavoring. That such augmentation of the recipes was being practiced was verified by conversations with State and local health department personnel in several parts of the country. Recognizing that of these additives, those containing proteins and fats, particularly protein, could easily make the most bacteriologically safe synthetic filling suitable for staphylococcal growth, we investigated briefly the effect of the addition of a representative common food additive containing both. Commercial dry, whole milk was chosen for this purpose because our experience also indicated that the addition of milk improved the palatability and texture of the synthetics, thus providing a great temptation for employing milk for this purpose. Table 5 summarizes the results. All of the fillings containing milk supported profuse growth of the inherent flora as well as staphylococci. Significantly, no suppression of staphylococcal growth occurred to lend support to our feeling that the major factor in the failure of some cream fillings to support staphylococcal growth may be the absence of required nutritional factors and accessory effects exerted by the pH and concomitant flora.

In view of these results, we briefly considered the effects of inadvertent contamination of a synthetic filling by a food substance that can commonly be introduced by mixing spoons, utensils, and similar equipment. Accordingly, the amount of beaten whole egg that would cling to a mixing spoon after drainage of excess egg

Table 6. Multiplication of *Staphylococcus aureus* in cream filling A containing 0.09 gm. of raw whole egg per 100 gm. of synthetic cream filling

Inoculum per gram at 0 hours		Number of staphylococci per gram after incubation of cream filling at room temperature		
Estimated	Actual	24 hours	48 hours	72 hours
10 ³ -----	8. 8 x 10 ²	1. 2 x 10 ³	7. 5 x 10 ²	5. 4 x 10 ²
10 ⁴ -----	1. 1 x 10 ⁴	1. 3 x 10 ⁴	7. 7 x 10 ³	4. 6 x 10 ³

was determined by successive weighings. This amount, 0.09 gm., was added to 100 gm. of filling A that had been prepared with water. Staphylococci growth results are presented in table 6. A very slight increase from the initial inoculums of about 10³ and 10⁴ staphylococci per gram was noted in 24 hours. The count then decreased by the end of 72 hours of incubation. The reduction in staphylococcal population was considerably less in the egg-filling combination than in filling A that contained no egg at the 10³ (table 2) and 10⁴ (table 4) inoculum levels. The results imply that minute amounts of natural food have a protective effect on the staphylococci in the synthetic fillings. Larger amounts of such additives, also added without design, conceivably could allow even the most suppressive cream filling to support staphylococcal growth when held at room temperature.

Growth of bacteria in finished pies. The results in the finished pies show considerable vari-

Table 5. Multiplication of *Staphylococcus aureus* and normal bacterial flora of synthetic cream fillings prepared with reconstituted commercial whole milk powder

Cream filling	Bacterial counts per gram after incubation at room temperature					
	Uninoculated		Inoculated with staphylococci			
	0 hours	72 hours	0 hours	24 hours	48 hours	72 hours
A-----	1. 7 x 10 ³	1. 1 x 10 ⁸	1. 2 x 10 ³	4. 9 x 10 ⁷	1. 0 x 10 ⁹	1. 3 x 10 ⁹
B-----	1. 0 x 10 ²	1. 6 x 10 ⁸	1. 1 x 10 ³	1. 3 x 10 ⁷	7. 7 x 10 ⁸	1. 0 x 10 ⁹
C-----	3. 8 x 10 ¹	1. 5 x 10 ⁶	1. 2 x 10 ³	5. 9 x 10 ⁵	1. 7 x 10 ⁸	6. 1 x 10 ⁸
D-----	5. 4 x 10 ¹	4. 1 x 10 ⁷	8. 2 x 10 ²	6. 9 x 10 ⁸	1. 0 x 10 ⁹	4. 6 x 10 ⁸
E-----	1. 0 x 10 ²	1. 2 x 10 ⁸	1. 1 x 10 ³	2. 0 x 10 ⁸	4. 7 x 10 ⁸	6. 5 x 10 ⁸
F-----	9. 4 x 10 ¹	4. 6 x 10 ⁸	1. 3 x 10 ³	8. 4 x 10 ⁷	1. 2 x 10 ⁹	1. 9 x 10 ⁹
G-----	5. 4 x 10 ¹	3. 3 x 10 ⁸	8. 9 x 10 ²	3. 0 x 10 ⁸	8. 3 x 10 ⁸	8. 6 x 10 ⁸

ation in the initial count (table 7). This is largely caused by variations in the amount of crust removed with filling for blending to prepare serial dilutions, despite attempts to remove representative portions of crust. The inoculum was added on the basis of the weight of filling alone, and the weight of crust tended to reduce the initial count per gram. In every instance a great increase in the staphylococcal counts occurred in 24 and 48 hours. Multiplication proceeded at a much slower rate by 72 hours, or a slight decrease in count occurred. The normal flora, largely bacilli, also increased greatly during the incubation period. Bacterial multiplication was greatest in filling F, which was prepared with milk according to instructions.

Organoleptically, the finished pies were considered to be only slightly spoiled at 24 hours, off odors being barely detectable. Spoilage was apparent by 48 hours, at which time some liquefaction in several fillings was apparent. Ordinarily, the pies would be unacceptable at this stage, an indication that consumption would be unlikely and the food-poisoning hazard would be avoided. Sufficient staphylococci were present at the end of 24 hours at room temperature, however (table 7), to constitute a definite hazard; it is likely that toxin could develop earlier when visible deterioration would not be apparent. The results suggest the possibilities of the addition of nutritional factors in the crust that are lacking in the fillings, alteration of the pH at the crust-filling interface, or reversal of the inhibitory balance exerted by the concomitant flora on staphylococci.

Summary

Seven synthetic cream fillings were examined for their ability to support multiplication of added *Staphylococcus aureus* (enterotoxigenic strain) and their normal flora at room temperature. When prepared with water, all seven supported multiplication of the bacteria present, largely bacilli; in two staphylococci decreased in numbers markedly; in three others the staphylococci decreased slightly; and two supported significant staphylococcal multiplication during incubation for 72 hours.

Increasing the number of the staphylococci in the inoculum and neutralizing the pH of the fillings did not result in significant staphylococcal multiplication but increased the ability of the staphylococci to survive in larger numbers in the product.

Substitution of milk for water in preparing the fillings, addition of minute amounts of whole egg and combination with pie crusts increased the ability of the fillings to support staphylococcal multiplication. Pies made with synthetic fillings rehydrated only with water supported profuse staphylococcal growth to the extent that they may be hazardous when held at room temperature before being sold.

Conclusions

The claims that synthetic cream fillings do not support bacterial growth at room-holding temperatures cannot be considered valid in connection with the preparation of completed pies. In fillings made with water only, staphylococci either increased in numbers or decreased, but

Table 7. Multiplication of normal flora and added *Staphylococcus aureus* in finished pies made with commercial pie crust mix and synthetic cream fillings

Filling	Bacteria per gram after incubation at room temperature					
	Uninoculated		Inoculated with staphylococci			
	0 hours	72 hours	0 hours	24 hours	48 hours	72 hours
A-----	110	1.3 x 10 ⁸	6.1 x 10 ²	1.0 x 10 ⁷	4.9 x 10 ⁷	8.8 x 10 ⁷
B-----	16	1.5 x 10 ⁸	1.3 x 10 ³	2.2 x 10 ⁷	1.0 x 10 ⁸	9.4 x 10 ⁷
C-----	30	1.9 x 10 ⁷	9.6 x 10 ²	6.6 x 10 ⁵	6.7 x 10 ⁷	8.4 x 10 ⁷
D-----	6	2.0 x 10 ⁷	1.7 x 10 ²	2.2 x 10 ⁶	7.2 x 10 ⁷	1.3 x 10 ⁸
E-----	182	1.3 x 10 ⁸	2.0 x 10 ²	6.3 x 10 ⁶	9.4 x 10 ⁶	1.3 x 10 ⁷
F-----	308	6.5 x 10 ⁸	1.3 x 10 ³	1.6 x 10 ⁸	4.7 x 10 ⁸	3.4 x 10 ⁸
G-----	22	3.2 x 10 ⁷	3.0 x 10 ²	1.2 x 10 ⁶	2.1 x 10 ⁷	3.7 x 10 ⁷

remained viable, thus constituting a potential hazard. The addition of nutrients containing protein or fat, or both, such as milk, egg white, shortening, or chocolate, either by design or inadvertently in the course of kitchen or bakery operations, is likely to improve the ability of the fillings to support profuse staphylococcal growth, or at least sustain larger numbers of viable staphylococci. Combining the cream filling with pie crust creates a situation in which staphylococci grow profusely within the time that completed pies may be held at room temperature before being sold. Therefore, pies made with synthetic fillings should be handled in the same manner as other pies, that is, under optimal conditions of cleanliness and refrigeration at 45° F. or below, during transportation and storage.

REFERENCES

- (1) American Public Health Association: Standard methods for the examination of dairy products. Ed. 11. New York City, 1960, p. 60.
- (2) Angelotti, R., Wilson, E., Foter, M. J., and Lewis, K. H.: Time-temperature effects on salmonellae and staphylococci in foods. I. Behavior in broth cultures and refrigerated foods. Technical Report F59-2. U.S. Department of Health, Education, and Welfare, Cincinnati, Ohio, 1959.
- (3) Peterson, A. C., Black, J. J., and Gunderson, M. F.: Staphylococci in competition. III. Influence of pH and salt on staphylococcal growth in mixed populations. *In* Bacteriological Proceedings, American Society for Microbiology, 1962, p. 33.
- (4) Peterson, A. C., Black, J. J., and Gunderson, M. F.: Staphylococci in competition. II. Effect of total numbers and proportion of staphylococci in mixed cultures on growth in artificial medium. *Appl Microbiol* 10:23 (1962).

Baltimore's Outdoor Health Fair



In 1963 for the third summer an outdoor health fair drew thousands of residents of an urban renewal area of Baltimore to a park in the neighborhood to receive "Keeping Well" health messages and various health services.

Co-sponsored by the Harlem Park Neighborhood Council and the Baltimore City Health Department, the fair has grown to encompass X-ray services, vision screening, immunizations for children, displays, and exhibits by almost every official and voluntary agency in Baltimore dealing with health and welfare services. Parades with health posters and flags, lectures, films for adults and children, demonstrations, and radio and television personalities were also part of the program.

Since the fair was held in an area of many low-income families, a booth demonstrating preparation of surplus foods was a popular feature. Many residents also stopped at the rat control exhibit to learn from the sanitarians how they could combat neighborhood pests.